Plant Growth-Promoting Bacteria Recovered from Winery Wastes

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Introduction and aims

In 2024, Portugal ranked among the world's top ten wine producers (OIV, 2025). This industry generates several by-products including grape stalks and sludge from the water used to wash winemaking machinery. Both residues are undervalued, as they are often burned, a practice that is economically and environmentally unsustainable.

A promising alternative is to reuse these wastes through composting. This controlled microbial driven process enriches the organic fraction of these residues, transforming them into a stable compost comparable to soil humus. Moreover, isolating Plant Growth-Promoting Bacteria (PGPB) from composted wine residues represents a valuable strategy, as both PGPB and compost may enhance plant growth and soil health. This study therefore aimed to isolate, characterize, and identify bacteria from different composting piles and assess their ability to produce plant growth-promoting (PGP) substances.

Methodology

Grape stalks + Sludge from winemaking machinery

Composting piles with sludge:stalks; w:w

Pile 1 → 3:1
Pile 2 → 2:1
Pile 3 → 1:1

after 4 months

Isolation of 74 bacterial isolates

Gram staining
Cytochrome oxidase
Catalase activities

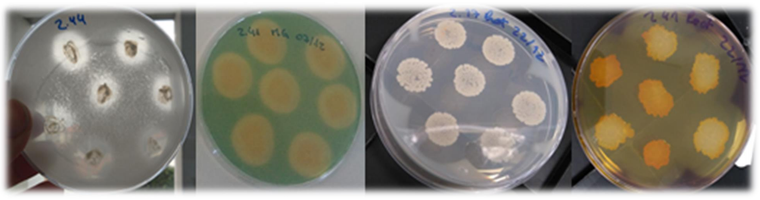
Screening for PGP substances:

P solubilization
N_2 fixation
Siderophores
Extracellular enzymes production
Indol-3-acetic acid (IAA) production
16S rRNA sequencing

Bacterial fingerprinting (Box-PCR)

23 bacterial isolates

Results



From left to right - Phosphate solubilization I Siderophores and extracellular enzymes production (protease and pectinase respectively).

Results

Table 1 – Characterization and identification of the bacterial isolates retrieved from the 3 composting piles.

Pile number	Isolate code	Catalase	Oxidase	Identification	% similarity
1	**1.32**	+	+	*Bacillus* sp. (in: firmicutes)	99%
	1.38	+	+	Not identified	
	1.39	-	+	*Cytobacillus* sp. strain R3112	98%
	2.1	+	+	*Bacillus sp.*	96%
	2.11	+	+	*Flavobacterium* sp. enrichment culture clone O1	98%
2	**1.22**	+	-	*Bacillus albus* strain PJAL1.1	99%
	1.27	+	+	*Bacillus paranthracis* strain CJB-H6	100%
3	**1.3**	+	+	*Cytobacillus horneckiae* strain UFLA WFC716	98%
	1.4	+	+	*Bacillus sp.*	94%
	1.5	+	+	*Bacillus* sp. (in: firmicutes)	95%
	1.7	+	+	*Bacillus* sp. (in: Bacteria) strain Cedo6	98%
	1.12	+	+	*Niallia sp.*	95%
	1.16	+	+	*Bacillus* subtilis strain LB16	97%
	1.17	-	+	*Cytobacillus* sp. strain R3112	97%
	2.26	+	-	*Bacillus* sp. (in: Bacteria) strain Fito_F7	98%
	2.29	+	+	*Agrobacterium pusense* strain FA62	99%
	2.30	+	+	*Agrobacterium tumefaciens* strain A38	99%
	2.32	+	+	*Pseudomonas trivialis* strain 4G761	99%
	2.37	+	+	*Pseudomonas fluorescens* strain B.xNS6	99%
	2.38	+	+	*Pseudomonas gessardii* strain ST3SE	99%
	2.40	+	+	*Pseudomonas putida* strain MT178	99%
	2.43	+	+	*Pseudomonas kurunegalensis* strain ANKC.G2	98%
	2.44	+	+	*Pseudomonas hunanensis* strain ZJTR9	99%

(–) negative production, **(+)** positive/weak production.

Table 2 – Plant growth-promoting (PGP) traits of the bacterial isolates retrieved from the 3 composting piles: P solubilization – phosphate solubilization, siderophore production, N_2 fixation – nitrogen fixation, production of Indol-3-acetic acid (IAA) and extracellular enzymes (protease, cellulase, pectinase and lipase).

Pile number	Isolate code	P solubilization	Siderophore production	N_2 fixation	IAA (µg ml^{-1})	Extracellular enzymes			
						Protease	Cellulase	Pectinase	Lipase
1	**1.32**	-	+++	++	33.9 ± 6.16	-	+	+	-
	1.38	-	+	+	**59.8 ± 0.29**	+	+	-	-
	1.39	+	++	-	44.3 ± 4.04	+	+	-	+
	2.1	+	+++	-	34.1 ± 3.28	+	+	-	-
	2.11	-	+++	++	29.1 ± 2.17	-	-	-	+
2	**1.22**	+	++	++	**48.5 ± 8.66**	+	+	-	+
	1.27	+	-	++	**46.3 ± 4.07**	+	+	-	-
3	**1.3**	+	+	-	**45.5 ± 8.83**	+	-	-	-
	1.4	+	+++	++	41.0 ± 8.99	-	+	-	-
	1.5	-	++	++	29.8 ± 3.93	+	-	+	-
	1.7	+	+++	-	**49.7 ± 4.03**	+	-	-	+
	1.12	-	++	++	28.4 ± 0.66	+	+	-	+
	1.16	+	++	+	23.8 ± 3.11	+	-	+	+
	1.17	+	++	-	38.9 ± 0.54	+	-	-	+
	2.26	+	-	++	44.8 ± 2.64	+	+	-	+
	2.29	-	+	++	**126.0 ± 5.42**	+	-	-	+
	2.30	-	+	++	**108.2 ± 3.05**	+	+	+	+
	2.32	+	+	-	**47.6 ± 3.56**	+	+	-	+
	2.37	+	+++	++	**45.0 ± 4.02**	+	+	-	+
	2.38	+	++	-	33.5 ± 0.38	+	+	-	+
	2.40	+	++	++	38.4 ± 3.97	-	+	+	+
	2.43	+	++	-	42.1 ± 3.18	+	+	-	+
	2.44	+	+++	++	32.4 ± 1.22	+	-	+	+

(–) negative production, **(+)** positive/weak production, **(++)** intermediate production, **(+++)** strong production.

- ✓ Six bacterial strains presented multiple plant growth-promoting traits (identified as *Bacillus* and *Pseudomonas);*
- ✓ IAA synthesis appears to be higher in the isolates identified as *Agrobacterium*, *Bacillus* and *Pseudomonas.*

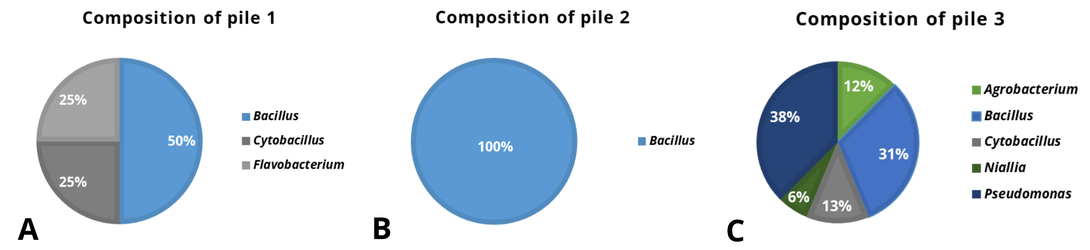


Figure 1 – Identification of the bacterial strains found in the composting piles according to their bacterial genus. 4 (figure 1A), 2 (figure 1B) and 16 isolates (figure 1C) were identified respectively on pile 1, 2 and 3.

- ✓ The different proportions of grape stalks and sludge seemed to influence the diversity of bacterial genera identified in each composting pile.

Conclusions

- The composting of wine-industry sludge and wine stalks facilitated the isolation and characterization of diverse bacterial strains possessing multiple plant growth-promoting traits.
- Composting pile 3, composed of equal proportions of grape stalks and sludge, allowed the characterization and identification of a higher diversity of isolates, corresponding to five distinct bacterial genera.

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